SECTION II
REMARKS

Regarding the Amendments

No claims have been amended by the present Response.

Thus, upon entry of the Response, claims 1-30 and 32-35 will remain pending, of which claims 1, 3-7, 10-30 and 33-35 are withdrawn.

Objection to the Abstract

The examiner has objected to the abstract of the application, because it has not been submitted on a separate page. Applicants respectfully draw the examiner's attention to the application, as filed, where a cover sheet was provided from Publication WO 2005/052135 of PCT application PCT/KR2004/001210 to which the present application claims priority, with an abstract on the front page of the publication. However, the abstract was not provided on a separate page.

Accordingly, the specification has been amended in the above Section I - Amendments to the Specification to include an abstract on a separate page, following page 29 of the claims. Additionally, the abstract has been amended to be less than 150 words.

As amended, the abstract is in accordance with the requirements of MPEP 608.01and 37 C.F.R. §1.71. Withdrawal of the objection is respectfully requested.

Rejection of Claims Under 35 U.S.C. §112

The withdrawal of the rejection of claims 2, 8, 9, and 32 under 35 U.S.C. §112, first and second paragraphs is acknowledged.

Rejection of Claims 2 and 32 Under 35 U.S.C. §103

In the Office Action mailed April 9, 2008, the examiner has rejected claims 2 and 32 under 35 U.S.C. §103(a) as unpatentable over Vemuri et al., *Appld. and Envrn. Microbiol.*, 2002, p. 1715-1727 (hereinafter Vemuri et al.) in view of Chang et al., *Appld.and Envrn. Microbiol.*, 1999, p. 1384-1389 (hereinafter Chang et al.). Applicants respectfully disagree.

Claims 2 and 32 of the application recite bacteria of *Mannheimia*, *Actinobacillus*, or *Anaerobiospirillum* mutated by disruption of the *ldhA*, *pfl*, *pta*, and *ackA* genes such that the mutant produces succinic acid under anaerobic conditions.

It is elemental law that in order for an invention to be obvious, the difference between the subject matter of the application and the prior art must be such that the subject matter as a whole would have been obvious at the time the invention was made to a person of ordinary skill in the art. In order to meet this standard for a proper §103 rejection, all claim limitations must be disclosed or derivable from the cited combination of references, there must be a logical reason to combine the cited references to produce an operable combination and there must be a reasonable expectation of success. See MPEP §2143:

> "**2143 Basic Requirements of a Prima Facie Case of Obviousness**
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> "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.
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> "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

In support of the rejection of claims 2 and 32 under 35 U.S.C. §103, the examiner cites Vemuri et al. as demonstrating "that lack of *ldhA*, *pfl* in *E. coli* strain NZN111 and *ldhA*, *pfl* and *pta* in *E. coli* strain AFP111 increase the production of succinic acid as [a] metabolic pathway for production of ethanol, lactate is disrupted." As acknowledged by the examiner, "Vemuri et al. does not teach disruption of *ackA* gene and more over said strain shows production of acetate."

Initially, the examiner's attention is respectfully drawn to Table 1 and line 12~14, col. 1, page 1716 of the Vemuri et al. reference, where the *E.coli* strain AFP 111 contains a disruption of the *ptsG* gene, not the *pta* gene, as cited by the examiner. "[T]he causative mutation in AFP111 was mapped to the *ptsG* gene, which encodes an enzyme of the PTS." The PTS acts on Step ① of the pathway of Fig. 4, as is stated in the description of Fig. 4. Therefore the PTS acts on the conversion of PEP to pyruvate. The *pta* gene mutated in the present invention, however, encodes

an enzyme of the phosphotrans acetylase. Accordingly, the Vemuri et al. reference lacks teaching of both *pta* and *ackA*.

The examiner cites Chang et al. as remedying the deficiencies of the Vemuri et al. reference, stating that "*Mannheimia, Actinobacillu* or *anaerobiospirillum* [citing present application]… and *E.coli* [citing Chang et al.]… have identical fermentation pathway for carboxylic acid metabolism" (Office Action mailed July 9, 2008, p. 4; emphasis added.) As such, the examiner draws the conclusion that disruption of *ldhA*, *pfl*, *pta*, and *ackA* would be obvious in *Mannheimia*, *Actinobacillus* and/or *Anaerobiospirillum*. Applicants respectfully disagree.

Contrary to the examiner's statement, the metabolic pathway for producing succinic acid in *Mannheimia*, *Actinobacillus* and *Anaerobiospirillum* is different from that in *E.coli*. The examiner's attention is respectfully drawn to the Zeikus et al. reference, cited in the IDS filed on February 20, 2008. In Zeikus et al. the metabolic pathways for producing succinic acid of the genus *Actinobacillus* and the genus *Anaerobiospirillum* are shown as a PEP carboxkinase pathway, while *E. coli* has a different pathway (Zeikus et al., p. 548; Table 1.).

Additional references cited in the IDS filed on February 20, 2008 (*see* Van der Werf et al., Laivenieks et al., Samuelov et al., and Kim et al.) demonstrate that the genus *Mannheimia*, the genus *Actinobacillus* and the genus *Anaerobiospirillum* of claim 2 have the same succinate pathway.

Furthermore, Chang et al., at Table 2 (reproduced below) shows that the effect of *pta* gene deletion in *E.coli* is very small. As such, the effect of *pta* deletion in *E.coli* greatly differs from the effect in the genus *Mannheimia*, the genus *Actinobacillus* and the genus *Anaerobiospirillum*. One of skill in the art would therefore not have been motivated to combine the teachings of the Vemuri et al. and Chang et al. references, as the effect in *Mannheimia*, *Actinobacillus* and *Anaerobiospirillum* would not have been expected.

TABLE 2. Fermentation balances of *E. coli* RR1 and its *pta* mutant[a]

Carbon source or product	Relative amt consumed or produced by:	
	RR1	JP200
Glucose (carbon source)	1.00 (13.1)[b]	1.00 (20.3)
Products		
Acetate	0.66	0.06
Ethanol	0.59	0.97
Lactate	0.26	1.52
Succinate	0.13	0.19
Pyruvate	0.03	0.02
Formate	1.13	0.03

Additionally, the wild type fermentation profiles of *E. coli* are different from those of the genus *Mannheimia*, the genus *Actinobacillus* and the genus *Anaerobiospirillum*. In the case of *Mannheimia*, *Actinobacillus*, and *Anaerobiospirillum*, those bacteria are cultivated while producing succinic acid under anaerobic conditions. However, *E. coli* cannot be simultaneously cultivated and produce succinic acid under anaerobic conditions. *E.coli* is cultivated under aerobic conditions, and it produces succinic acid as a minor fermentation product under anaerobic conditions. These characteristics are stated in the Lin et al. reference attached hereto as Exhibit A:

> "*E. coli* naturally produces succinate as a minor fermentation product under anaerobic conditions [but]…[u]nder aerobic conditions, succinate is not produced as a by-product in *E. coli* and acetate is the main by-product." (Lin et al., p. 148, col. 2).
>
> "Anaerobic conditions often cause poor cell growth and slow carbon throughput, and therefore low production rates…[s]trategies to overcome the anaerobic barrier have included generating enough biomass under aerobic conditions, then switching to anaerobic conditions for succinate production." (Lin et al., p. 149, col. 1; emphasis added).

Vemuri et al. in view of Chang et al. therefore fails to provide any derivative basis for the claimed invention. The combination of references fails to show a mutant containing *ldhA*, *pfl*, *pta* and *ackA* mutations that produces succinic acid under anaerobic conditions. Additionally, there would have been no logical reason for one of skill in the art to combine such references, as the wild type fermentation profiles of *E. coli*, *Mannheimia*, *Actinobacillus* and *Anaerobiospirillum* differ and, therefore, the mutant profiles would not expected to be predictive of one another. Accordingly, no basis of *prima facie* obviousness of the claimed invention is presented by such cited references.

As Vemuri et al. in view of Chang et al. does not provide any logical basis for the bacterial mutants recited in claims 2 and 32, Vemuri et al. in view of Chang et al. does not render the claimed invention obvious. Accordingly, withdrawal of the rejection of claims 2 and 32 under 35 U.S.C. § 103 (a) as being obvious over Vemuri et al. in view of Chang et al. is respectfully requested.

CONCLUSION

Based on the foregoing, all of Applicants' pending claims 2, 8, 9, and 32 are patentably distinguished over the art, and are in form and condition for allowance. The Examiner is requested to favorably consider the foregoing and to responsively issue a Notice of Allowance.

The time for responding to the April 9, 2008 Office Action without extension was set at three months, or July 9, 2008. This Response is therefore timely and no fees are believed to be due for the filing of this paper. However, should any fees be required or an overpayment of fees made, please debit or credit our Deposit Account No. 08-3284, as necessary.

If any issues require further resolution, the Examiner is requested to contact the undersigned attorney at (919) 419-9350 to discuss same.

Respectfully submitted,

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Encl.
Exhibit A [10 pgs.]

The USPTO is hereby authorized to charge any deficiency or credit any overpayment of fees properly payable for this document to Deposit Account No. 08-3284